Nasal Cytology in Southwest Metropolitan Mexico City Inhabitants: A Pilot Intervention Study

Lilian Calderon-Garcidueñas[1] *and Guadalupe Roy-Ocotla*[2]

[1]Centro Nacional de Investigación en Calidad Ambiental Cenica, Mexico City, Mexico;
[2]Centro de Ciencias de La Atmosfera, National University of Mexico, Mexico City, Mexico

Southwest metropolitan Mexico City (SWMMC) inhabitants have been exposed several hours per day for the last 6 years to photochemical smog, ozone being the most important oxidant pollutant. Subjects exposed to the SWMMC atmosphere develop several histopathological changes in their nasal mucosa: dysplasia is the most significant, affecting 78.72% of adult individuals within 60 or more days of residence in SWMMC. This study was originally designed to explore whether chemical intervention could modify nasal dysplasia, as determined by nasal cytology, in a defined adult population. In a placebo-controlled, randomized, double-blind trial, 177 healthy male subjects were divided into 5 groups to whom 5000 IU of vitamin A, 100 IU of vitamin E, a combination of vitamins A and E (5000 IU + 100 IU), 16 mg of ß-carotene, or placebo were administered daily for 4 months. Sixteen clinical and cytological variables were monitored. No effect on dysplasia was seen at the end of the 4-month trial; however, an apparent reversibility as well as progression of the dysplastic nasal lesions and high correlation coefficients between dysplasia and nasal cytology of polymorphonuclear leukocytes (PMNs; 0.85), squamous metaplasia (SM; 0.50), and nasal mucosa atrophy (NMA; 0.41) were found. A mathematical theoretical nasal dysplasia (*tD*) predictor equation for SWMMC adult male inhabitants is proposed ($tD = 0.85\ \Delta PMNs + 0.50\ \Delta SM + 0.41 \Delta NMA + 0.98$), in which PMNs are the best single dysplasia predictor, and all variables are independent. We suggest that the nasal cytological changes in SWMMC inhabitants may constitute an adaptative response to environmental pollutants, and long-term follow-up of these subjects will be necessary to establish the possible outcomes of the nasal abnormalities. **Key words**: intervention study, nasal mucosa cytology, ozone, photochemical smog. *Environ Health Perspect* 101: 138–144(1993)

Metropolitan Mexico City (MMC) is one of the most polluted urban areas in the world, and southwest metropolitan Mexico City (SWMMC) cannot absorb large amounts of pollutant emissions without exceeding health-based air quality standards. We previously reported the development of nasal mucosa dysplasia in 17.64% and 78.72% of <30-day and >60-day SWMMC residents and suggested that ozone, the most important photochemical pollutant in SWMMC, could play a synergistic role in combination with other toxic and potential carcinogens (*1*). The present study was designed to explore if chemoprevention, both secondary and tertiary (*2*), is capable of halting or delaying the development of nasal mucosa dysplastic lesions in SWMMC inhabitants. Accordingly, a randomized, double-blind, placebo-controlled study using low oral doses of vitamin A (5000 IU), vitamin E (100 IU), vitamins A and E (5000 IU and 100 IU), and ß-carotene (16 mg) was undertaken.

The choice of both vitamins and precursor was made on the basis of previous reports (*2*). Briefly, vitamin A allows normal growth and epithelial differentiation, and in numerous studies vitamin A and ß-carotene have been used to induce remission and/or inhibition of the development of oral leukoplakias (*3–5*). In betel quid chewers, the clinical trials varied in length from 3, 6, and 14 months, and variable doses of vitamin A and/or ß-carotene were administered [vitamin A, 200,000 IU/ week/6 months (*3*), 100,000 IU/week/3 months (*4*), 60 mg/week/6 months (*5*); ß-carotene, 180 mg/week plus vitamin A 100,000 IU/ week/3 months (*4*)]. Interestingly, frequency of micronucleated buccal mucosa cells was the intermediate endpoint used to estimate the response of the oral mucosa to vitamin A or ß-carotene, with changes seen as early as 1–3 months, whereas modifications in the frequency of preneoplastic leukoplakias required 4–6 months. Epidemiological studies have shown a protective effect against certain cancers (lung, oral cavity, esophagus, stomach, breast, ovary, etc.) when the dietary intake of vitamin A or ß-carotene is significantly increased compared to controls (*2*). Retinoids are active in the promotional phase of carcinogenesis (*6*), and their chemopreventive actions are reversible upon withdrawal, a very important point for our SWMMC-exposed population, since theoretically it implies that the subjects have to continue with retinoids or their precursors for as long as they remain in the highly polluted urban environment. Because our final aim is to give the chemoprevention treatment for long periods of time to a large population, including women and children, the doses of vitamin A and ß-carotene chosen are low compared to those used in other clinical trials.

Vitamin E is a well-known antioxidant that acts in the promotional phase of carcinogenesis through its action on free radicals (*2,7*). Absence of dietary vitamin E exacerbates lung injury from ozone inhalation in rats (*8*). Elsayed et al. (*8*) found that the magnitude of this protective effect does not increase proportionately with increased dietary vitamin E supplementation beyond a certain level.

The results of our 4-month pilot trial show no statistically significant difference between the treated and placebo groups on the chosen endpoint of nasal dysplasia, remission of established nasal dysplasias and/or inhibition of the development of new dysplasias. Furthermore, we present evidence of a high correlation coefficient between nasal dysplasia and polymorphonuclear leukocytes (PMNs) revealed by nasal cytology and also of a direct relationship between an increment in PMNs in the nasal turbinates and the total number of monthly exposure hours to ozone above 0.11 ppm. A theoretical nasal dysplasia predictor equation is proposed for SWMMC adult males.

We suggest that the nasal cytological changes in SWMMC inhabitants may constitute an adaptative response to the urban

Address correspondence to L. Calderon-Garcidueñas, Cerro del Vigilante No. 96, Romero de Terreros, Coyoacan, 04320 Mexico DF Mexico.
We thank Humberto Bravo Alvarez from the Atmospheric Sciences Center, National University of Mexico and the Centro Nacional de Investigación en Calidad Ambiental (CENICA) for the pollutant SWMMC data and for important suggestions and support; Azucena Hernandez Martinez, Central Military Hospital, Mexico City, for cytological evaluations; and Hugo López López Naval Medical Center, for ENT examination assistance. Special thanks to Colin M. White Muller for careful review of this manuscript, to Ricardo Torres and Rodolfo Sosa for useful ideas, and to Martin Ayala Flores for computer support. We acknowledge the kind support of the Roche Co., Mexico, for supplying the vitamins and the ß-carotene.

environmental pollutants. A long-term follow-up of these subjects will be necessary to establish the possible outcomes of the nasal abnormalities.

Methods

The project was approved by the Medical Ethical Committee of the Naval Medical Center, and informed consent was obtained from all subjects. We selected 206 healthy male subjects between 18 and 50 years of age (mean 30.4 ± 7.2 years) for the study. The participants were health workers, medical staff, and Marines working in SWMMC, where they spend ≥ 8 hr (from 7:00 AM onward), 6–7 days per week. In addition, 49.75% of these subjects lived in the SWMMC urban area. We chose this population because of the many advantages it presented for an intervention project. The subjects were stationed in an SWMMC area for which we have atmospheric pollutant data 24 hr per day, all year long, for several years; their movements during the day (when the pollutant emissions are at their highest) were virtually restricted to a few city blocks; the participants had no known exposures to local sources of air pollutants; they could be readily reached to control the intake of medication; and, finally, they were very cooperative with researchers. Subjects were introduced into the study during November and December 1990, and the trial ended 4 months later.

Clinical data obtained included age, sex, place of residence within MMC, length of residence, occupation, personal history of allergic diseases, smoking and drinking habits, and otolaryngologic history (epistaxis, nasal dryness, nasal mucous, nasal obstruction, rhinorrea, and septal deviation). These latter six clinical variables form part of the clinico-cytological variables (CCV) and were scored as absent, minimal, moderate, or severe. Septal deviation was scored as absent or present. We excluded individuals with a history of surgical otolaryngologic procedures or in need of treatment, atopic rhinitis, asthma, upper respiratory infections, known job exposure to toxic substances, photocopying machine workers, cigarette smokers with a consumption of more than half a pack per week, subjects traveling to and from MMC, and those regularly taking vitamin preparations or undernourished.

The study was a double-blind randomized, placebo-controlled, 4-month trial, and the subjects were divided into 5 groups. All groups received daily doses of vitamin A (5000 IU), vitamin E (tocopheryl acetate; 100 IU), a combination of vitamins A and E (5000 IU + 100 IU), ß-carotene (16 mg), or placebo (vegetable oil). All participants were submitted to an ear–nose–throat examination by an otolaryngologist three times during the study period: days 0, 60, and 120. The ENT findings, upper respiratory symptomatology and medication side effects were recorded; the administration of the designated doses was closely monitored throughout.

Cytology Sampling

After the rhinoscopic examination, we scored the severity of the nasal mucosa findings by reference to five parameters (CCV); atrophy, hyperemia, hyaline mucous bridges, bleeding friable mucosa, and pale mucosa (1 = absent to 4 = severe). The nasal cytology specimen was taken by the same physician from behind the anterior curvature of the inferior turbinate of the nasal cavity with the best airflow passage. The second and third samples were obtained from the same side as the original one. A small piece of a wooden tongue depressor was used to take the cytology sample. We obtained two slides from each subject, and Papanicolau-stained slides were prepared and examined by two pathologists separately,with no access to clinical information. The following cytological parameters were scored: ciliated respiratory-type cells, goblet cells, squamous metaplasia cells, inflammatory cells, and dysplasia (Figs. 1–4). For the first three parameters, the scoring system was 1 = absent, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, and 5 = >76%. The last two parameters were scored absent, mild, moderate, and severe. In 86% of the cases, the scores of both observers coincided; in the remaining cases, the samples were reexamined, and a joint determination of the appropriate score was reached. Only the results of the first and the third clinical and cytological evaluations are considered here.

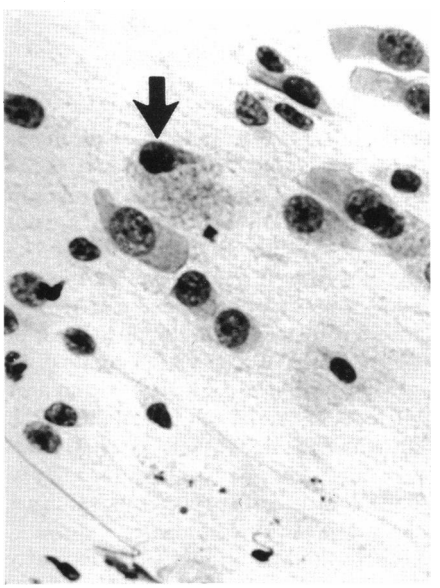

Figure 1. Nasal cytology smear taken from the inferior turbinate shows ciliated respiratory type-cells and goblet cells. The mucus-producing goblet cells (arrow) display a distended cytoplasm and a peripherally located nucleus. Papanicolau-stained; 200×.

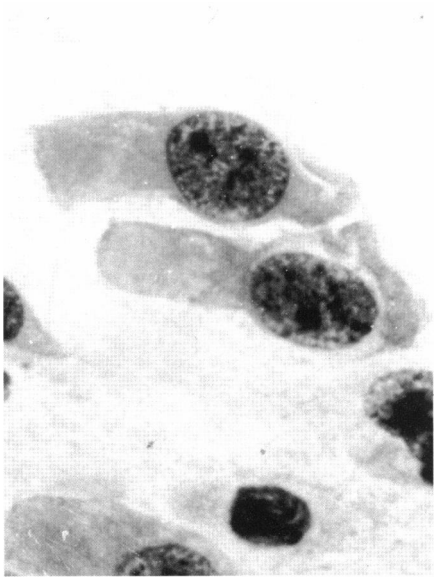

Figure 2. Respiratory cells in profile. Note the distal terminal plates with loss of cilia and the thin, whiplike, elongated proximal ends. The chromatin appears faintly granular. Oil immersion; ~1000×.

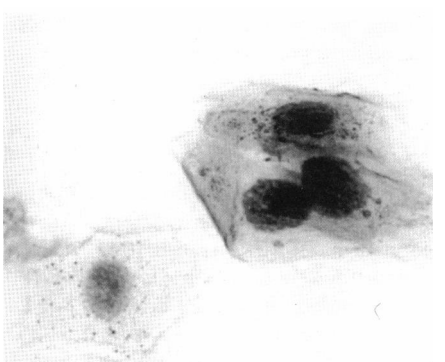

Figure 3. Cluster of cells. Note the three squamous metaplastic cells with mild atypia and an isolated, regular squamous metaplastic cell. Oil immersion; ~550×.

In this study, the endpoint for assessing chemical intervention was dysplasia: remission of established nasal dysplasias and/or inhibition of the development of new dysplasias. The criteria of cytological dysplasia was made on the bases of the identification of dysplastic cells exhibiting mild to severe pleomorphism, both in the size and shape of cells and their nuclei, as well as alterations in the staining characteristics.

Statistical Analysis

We used Student's *t*-test, analysis of variance with contrast, Fisher's least significant difference test, Cochran-Mantel-Haenszel

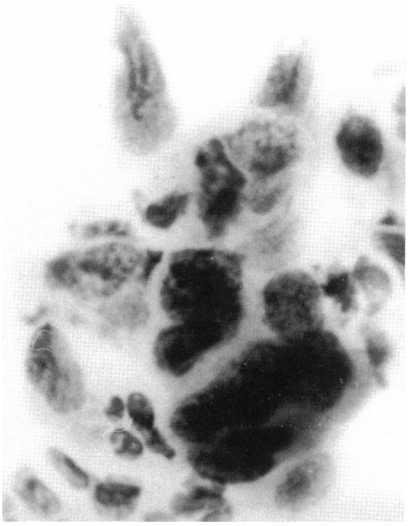

Figure 4. Cluster of markedly atypical cells. Note the large, irregularly shaped, densely hyperchromatic nuclei. Oil immersion; ~1000×.

chi-square test, and multivariate linear regression analysis for statistical analysis. For the development of the theoretical dysplasia predictor equation, multivariate linear analysis was used. Only $p < 0.05$ was considered significant.

Results

Air Quality Data

Ozone (O_3) has been monitored continuously at the Atmospheric Science Center (located in SWMMC) since 1979 and not until 1981 were diurnal levels of $O_3 > 0.11$ ppm recorded: 40 and 30 hr of $O_3 > 0.11$ ppm were recorded in 1984 and 1985, respectively. The number of hours SWMMC inhabitants are exposed to $O_3 > 0.11$ ppm has been steadily increasing since September 1986 and coincided with the marked decrease of tetraethyl lead in MMC gasoline. For 11 of the 12 months of 1990 and for every month in 1991, more than 85 hr of $O_3 > 0.11$ ppm were recorded, for a total of 1403 hr in 1990 and 1561 hr in 1991 (Table l). In fact, there were only 6 days in 1991 with diurnal levels of $O_3 < 0.11$ ppm. A similar trend has been observed in the monthly average of maximal concentrations of O_3 recorded in SWMMC (Table 2): during 1990 and 1991 the maximum levels were mostly above 0.2 ppm, with the highest levels (> 0.5 ppm) recorded in October 1991.

Concentrations of sulfur dioxide in SWMMC for the years 1990–1991 have shown a diurnal cycle with maxima in the morning hours and in the winter months; however, the EPA Air Quality Standard of 30 ppb annual average for sulfur dioxide is not exceeded in SWMMC. Formaldehyde levels in SWMMC show a peak shortly before noon, and in samples taken between

Table 1. Number of hours with ozone levels above 0.11 ppm, per month, 1987–1991, southwest metropolitan Mexico City

Month	1987	1988	1989	1990	1991
January	56	48	77	129	101[a]
February	33	92	57	85	115[a]
March	35	39	65	55	144[a]
April	21	64	113	104	131
May	48	77	144	177	186
June	46	51	133	118	139
July	31	93	93	106	134
August	88	66	100	126	142
September	99	104	54	98	84
October	116	111	139	121	119
November	71	104	145	139[a]	130
December	96	110	104	145[a]	136
Total	740	959	1224	1403	1561

[a] Intervention trial months.

Table 2. Monthly average of maximal concentrations of ozone (ppm), 1986–1991, southwest metropolitan Mexico City

Month	1986	1987	1988	1989	1990	1991
January	0.072	0.136	0.145	0.167	0.210	0.170[a]
February	0.060	0.110	0.190	0.141	0.180	0.200[a]
March	0.057	0.103	0.122	0.155	0.140	0.210[a]
April	0.065	0.113	0.168	0.190	0.220	0.200
May	0.055	0.133	0.164	0.220	0.280	0.252
June	0.039	0.122	0.134	0.250	0.200	0.220
July	0.043	0.122	0.184	0.190	0.200	0.230
August	NR	0.174	0.158	0.190	0.210	0.230
September	0.144	0.176	0.189	0.180	0.210	0.190
October	0.190	0.205	0.176	0.220	0.200	0.250
November	0.120	0.160	0.177	0.210	0.220[a]	0.220
December	0.123	0.170	0.189	0.170	0.220[a]	0.200

NR, ozone values not recorded.
[a]Trial months.

10:30 AM and 12:30 PM, the mean average is 0.021 ppm (25.66 $\mu g/m^3$; data from October 1987 to February 1988). In the study period from November 1990 to March 1991, the mean average of formaldehyde was of 0.03 ppm (44 $\mu g/m^3$).

Study Population

Of the initial 206 subjects, 177 were monitored throughout the 4-month trial; 29 subjects were excluded due to changes in work location. The participants had a mean length of residence in MMC of 165 ± 143 months (46% of them had < 72 months and 54% > 72 months residence in MMC); only 19 participants had less than 1 year of residence in MMC (5.94 ± 2.8 months).

There were no statistical differences among groups at day 0 in regard to clinical, otolaryngological, and nasal cytology findings. No attempt was made in this study to determine the indoor/outdoor exposure schedule or the level of physical exercise of the participants, although the majority spent most of their working time indoors and were in the category of intermittent light and moderate physical exercise. No medication side effects were reported.

We grouped the subjects in terms of their cytological dysplasia and their chemoprevention treatment (Table 3). Sixteen clinical and cytological variables were evaluated at days 0 and 120, and their score increments or decrements were determined at the end of the trial in this manner: $\Delta CCV = CCV_2 - CCV_1$, where ΔCCV represents the increment or decrement (> 0, < 0) between the two evaluations at the beginning and end of the trial. We then determined, by multivariated linear regression analysis, that 3 of the 16 CCVs studied had the highest correlation coefficients with cytological dysplasia: PMNs (0.76), SM (0.554), and NMA (0.515). These parameters were independent variables (correlation coefficients SM:PMNs, 0.162; SM:NMA, 0.085; PMNs; NMA 0.01660). The distribution of individual subjects by cytological dysplasia, chemoprevention treatment, and decrements or increments in the three important variables that correlate with dysplasia is available upon request. The sum of the increments or decrements in PMNs, SM, and NMA was designated "expected dysplasia" (*eD*). The response rate of the four treated groups versus the placebo was evaluated in terms of cytological dysplasia at the end of the trial, and no statistically significant difference was found; data in Table 3 were assessed by analysis of variance (F test, F_o 0.0199 < 2.87 and F_1 0.89 < 2.84). Nor was there any difference in cytological dysplasia when we analyzed the data, regardless of treatment: At day 0, 91 subjects had dysplasia [51.41%: 72 (79.12%) mild, 19 (20.87%) moderate] and at day 120, 90 subjects had dysplasia [51%: 75 (83.33%) mild, 13 (14.44%) moderate, and 2 (2.22%) severe].

Most subjects, regardless of treatment, showed an increased severity of squamous metaplasia ($p < 0.01$), nasal mucosa atrophy ($p = 0.012$), mucosal hyperemia, a decrease in the numbers of ciliated respiratory-type cells, and a decrease in the amount of nasal mucus ($p = 0.0073$). There were no changes in epistaxis and bleeding friable mucosa. A statistically significant difference in nasal symptomatology was present in the group receiving vitamin A in regard to nasal dryness ($p = 0.01372$) and nasal obstruction ($p = 0.02155$) and in the group receiving ß-carotene in regard to nasal dryness ($p = 0.0072$). On the other hand, the placebo group showed a significant worsening in nasal dryness and nasal obstruction ($p < 0.01$). No differences were seen in terms of dysplasia or CCVs between nonsmokers and smokers ($p = 0.267$). The 19 subjects with less than 1 year of residence in MMC showed a high correlation coefficient between squamous metaplasia and decreased nasal mucus (0.60) and between length of residence in months and expect-

Table 3. Subjects groups in terms of cytological dysplasia, chemoprevention treatment, and length of residence in Mexico City

Treatment groups[a]	Dysplasia group[b]						No. of patients
	GI	GII	GIII	GIV	GV	GVI	
G1	11	7	7	10	0	2	37
G2	5	7	9	11	0	2	34
G3	10	9	7	4	1	0	31
G4	5	4	13	11	1	2	36
G5	10	8	8	10	1	2	39
Residence							
>1 year	40	32	39	38	2	7	158
<1 year	1	3	5	8	1	1	19
Total no. of patients	41	35	44	46	3	8	177

[a]GI, vitamin E; G2, vitamin A; G3, vitamins A and E; G4, ß-carotene; G5, placebo.
[b]GI, subjects without dysplasias throughout the study; GII, subjects with dysplasias that did not change throughout the study; GIII, subjects who went from no dysplasia to dysplasia at the end of the study; GIV, subjects who started with dysplasia, but had no dysplasia at the end of the study; GV, subjects who went from a low degree of dysplasia to a higher degree of dysplasia; GVI, subjects who went from a high degree of dysplasia to a lower degree.

ed dysplasia (0.51). Furthermore, only 1 of the 19 newly arrived subjects showed no dysplasia during the 4-month observation period; the remaining 18 individuals occupied a place in each of the cytological dysplasia groups (Table 3).

Theoretical Nasal Dysplasia Predictor Equation

Based on the observation of high correlation coefficients between three CCVs and cytological dysplasia, the following equation was applied as a model:

$$uD \text{ (unadjusted dysplasia)} = 0.76\,\Delta\text{PMNs} + 0.55\,\Delta\text{SM} + 0.52\,\Delta\text{NMA} \quad (1)$$

where ΔPMNs, ΔSM, and ΔNMA represent the changes (increments or decrements) in these variables at the end of the 4-month observation period. Subsequently, the equation was written as follows:

$$tD \text{ (adjusted, theoretical dysplasia)} = K_1\,\Delta\text{PMNs} + K_2\,\Delta\text{SM} + K_3\,\Delta\text{NMA} + K_4 \quad (2)$$

where K_1, K_2, and K_3 are adjusted constants based on the original correlation coefficients between the pertinent CCV data, and K_4 is a proportionality constant; this leads to a proposed final form:

$$tD = 0.85\,\Delta\text{PMNs} + 0.50\,\Delta\text{SM} + 0.41\Delta\text{NMA} + 0.98. \quad (3)$$

Correlation coefficients for SM and NMA are lower after adjustment, while the value increased for PMNs. Figure 5 shows the *tD* scores versus increments or decrements over time of squamous metaplasia (ΔSM) and polymorphonuclear leukocytes (ΔPMNs).

We then analyzed *eD* and *tD* in relation to cytological dysplasia and determined that the majority of subjects without dysplasia throughout the study and subjects who started with dysplasia but who had no dysplasia at the end of the study correspond to negative or low positive score ranges (*eD*, -2–0 and *tD*, -0.28–1.50). Subjects with dysplasia that did not change throughout the study are in the middle of the scale, and subjects who went from no dysplasia to dysplasia at the end of the study correspond to the highest score ranges (*eD*, 3,4 and *tD*, >2.51). Theoretical dysplasia scores in relation to cytological dysplasia are shown in Table 4. Note that there are a certain number of subjects who display high *tD* scores in spite of the absence of cytological dysplasia (the 13 subjects in GI with *tD* values between 1.51 and 2.50), and the GII individuals with dysplasia that did not change throughout the study had *tD* values of 2.51–3.50 (n = 9). The implications of these findings are complex. On one hand, this relationship between cytological dysplasia and low or high *tD* scores could indicate the predictive value of the equation; namely, subjects without cytological dysplasia after two evaluations but with high *tD* scores are at risk of developing cytological dysplasia in the future, and the subjects with a steady dysplasia perhaps will go into a higher severity of dysplasia if their *tD* values remain high over a period of time. This prediction must be confirmed by periodical cytological exams in the same subjects. On the other hand, we have to take into account the inherent flaw in cytological procedures; that is, the presence of false positives or negatives. Long-term follow-up of these individuals will clarify these issues.

It is interesting to point out here that the correlation coefficient between resi-

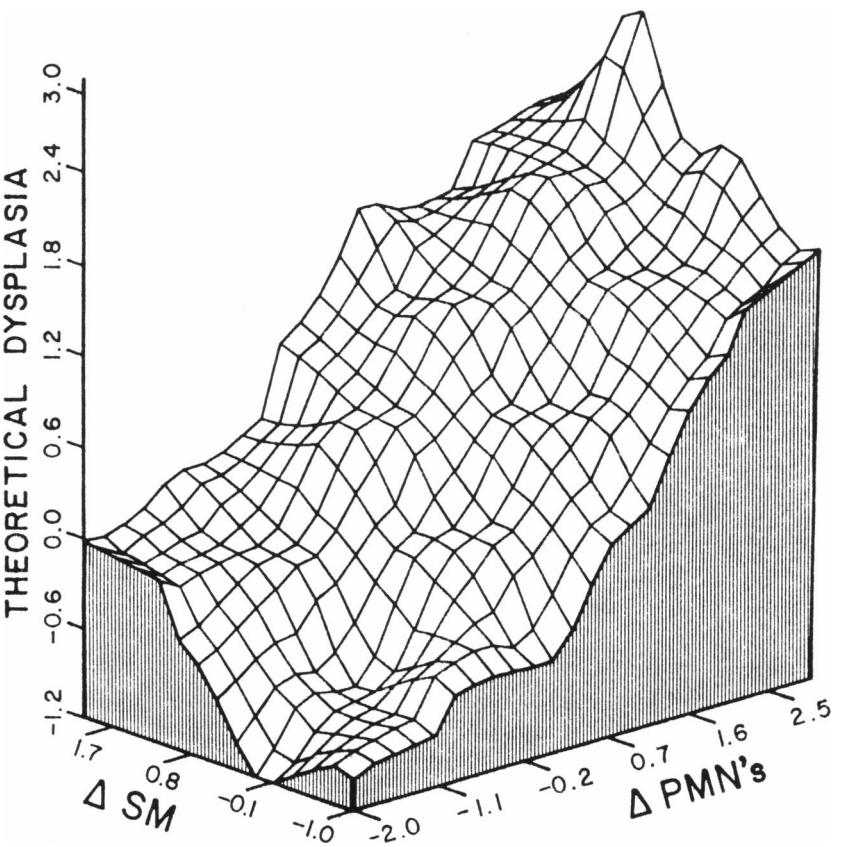

Figure 5. Theoretical dysplasia (*tD*) scores versus increments or decrements over time of squamous metaplasia (ΔSM) and polymorphonuclear leukocytes (ΔPMNs). Data based on the equation: $tD = 0.85\,\Delta\text{PMNs} + 0.50\,\Delta\text{SM} + 0.41\,\Delta\text{NMA} + 0.98$.

Table 4. Theoretical dysplasis (*tD*) scores and cytological dysplasia groups

tD	Dysplasia group[a]						No. of patients
	GI	GII	GIII	GIV	GV	GVI	
-0.28–0.54	2	4	4	12	0	3	25
0.55–1.50	25	7	6	22	1	3	64
1.51–2.50	13	15	14	5	2	2	51
2.51–3.50	1	9	19	5	0	0	34
>3.51	0	0	1	2	0	0	3

[a]See Table 3 for a description of dysplasia groups.
Higher scores are seen in subjects with persistent dysplasia (GII) and for subjects who went from no dysplasia to dysplasia at the end of the study (GIII).

dence in months and *tD* scores in the group of 19 subjects with less than 1 year of residence in MMC was 0.66. This relationship holds true for all subjects who had lived less than 20 months in MMC; it did not hold true, however, for individuals with more than 10 years in MMC (data not shown). Further, 65% of subjects with high *tD* scores had lived less than 72 months in MMC, whereas 61% of subjects with low scores had lived more than 72 months in MMC. The 72-month division is crucial because it marks the abrupt change in diurnal ozone values in SWMMC (Table 2).

In an attempt to correlate PMN values for the population studied with actual O_3 values during the study period, we established two points: P_2 (x = 128.8, y = 48), where the value on the abscissa corresponds to the average number of hours with O_3 > 0.11 ppm over the 5-month study period (November 1990 through March 1991; see data in Table 1) and the value on the ordinate corresponds to the percentage of patients at the end of the study with ΔPMNs > 0 (48%) and P_1 (x = 113.8, y = 42.42), where 113.8 corresponds to the average number of hours with O_3 > 0.11 ppm during the 5 months before the study (June through October 1990), and 42.41 represents the percentage of subjects living in SWMMC assumed to have developed an increment in PMNs, based on the P_2 value. The straight line P_1–P_2 can be prolonged, and we established a presumptive O_3 value when 50% of the SWMMC population has an increment of PMNs in their nasal turbinates; this value corresponds theoretically to 130 hr in a month with ozone values above 0.11 ppm (a rather common situation in SWMMC).

Discussion

This study is the first double-blind, placebo-controlled trial with low oral doses of vitamins A, E, and ß-carotene administered to individuals chronically exposed to photochemical smog, of which ozone is the most important constituent. Nasal dysplasia was the chosen endpoint of the study.

As reviewed by Bertram et al. (*2*), vitamin A and retinoids allow normal growth and epithelial differentiation and have well-demonstrated effects on the inhibition of experimental and human carcinogenesis at several sites, including the oral cavity (*3–5*). In a population of betel quid chewers in India, a 6-month treatment with oral vitamin A (200,000 IU/week) induced remission of established oral leukoplakias in 57% of patients, preventing the formation of new leukoplakias within the trial period (*4*). Beta-carotene also has chemopreventive and antitumor activity (*4–6*). Both vitamin A and ß-carotene are active in the promotional phase of carcinogenesis; there is a delay in tumor onset with a slowdown in the progression to frank neoplasia (*2*). Vitamin E, on the other hand, is a lipid-phase antioxidant that inhibits cellular damage produced by oxygen free-radicals and is also active in the promotional phase of carcinogenesis.

The results of this study show no effect on dysplasia with any of the vitamins or the ß-carotene—neither remission of established nasal dysplasias nor inhibition of the development of new ones. Further, we found a worsening of squamous metaplasia in all patients, regardless of treatment, with only a few improved clinical parameters in treated subjects. The fact that no positive results were seen might be result of the short trial time and the lack of interim endpoints; positive effects on chemoprevention results will have to be studied in a larger number of subjects over a longer period. More importantly, however, this study allowed us to establish a relationship between nasal dysplasia and clinico-cytological variables that show increments in relation to high levels of ozone in the polluted SWMMC atmosphere.

Ozone injury involves the development of oxygen free-radical events, which are responsible for DNA strand breaks and the formation of base adducts such as 8-hydroxyguanine (*9*). Borek et al. (*10*), using *in vitro* transformation, reported that O_3 induced neoplastic transformation in primary hamster embryo cells and mouse fibroblast cultures, with enhanced levels of free-radical-mediated lipid peroxidation products. Borek at al. (*10*) concluded that "ozone acts as a direct carcinogen and co-carcinogen on susceptible cells, therefore having important consequences for public health."

The nasal cavity absorbs 40–70% of inhaled ozone in dogs (*11*) and is a major target for environmental pollutants. Exposure of rats to 0.8 ppm O_3 for 3 or 7 days results in hyperplasia of the nasal cuboidal transitional epithelium; the rat cuboidal transitional epithelium is the epithelial cell type most responsive to the effects of O_3 exposure (*12*). Johnson et al. (*12*) encountered a variable response to ozone depending on concentration, duration of exposure, and cell type; these authors suggest that there is an immediate proliferative response to ozone (within 3 days) which then subsides and is followed by the development of hyperplastic lesions. Similar changes are described in the transitional and respiratory nasal epithelia in the Bonnet monkey after 6 days of exposure to 0.15 ppm O_3 (*13*). Further, three 6-hr/day exposures to 0.8 ppm O_3 in rats triggered hyperplastic and metaplastic changes in the nasal nonciliated cuboidal epithelium (*14*). As reported earlier, basal cell hyperplasia and squamous metaplasia are seen in human nasal biopsies of subjects exposed for more than 2 months to levels of O_3 > 0.11 ppm (*1*). In the nasal biopsy study we observed in some cases an acute inflammatory intraepithelial infiltrate as well as marked proliferation of capillaries in or against basement membranes, and submucosal proliferation of thin vascular channels. Hotchkiss et al. (*15*) reported large numbers of pavementing neutrophils within nasal turbinate blood vessels in rats exposed to 0.8 ppm O_3 immediately and 18 and 66 hr after exposure, although they did not mention increased numbers of submucosal blood vessels. The same authors demonstrated that a single 6-hr exposure to O_3 (0.12 and 0.8 ppm) induces an inflammatory response within the rat nasal cavity; furthermore, the number of PMNs recovered by nasal and bronchoalveolar lavage (NAL and BAL), accurately reflected the inflammatory response in the nasal cavity and lungs injured by the acute ozone toxicity. Human exposures to 0.4 ppm O_3 for 2 hr elicit a 7.7-fold increase in PMN in NAL (immediately postexposure), and this increase is detectable 18 hr later (6-fold), both in NAL and BAL (*16*).

In the present study, the percentage of patients with PMNs in their nasal cavity, regardless of treatment, was 60% at day 0 and 81% at day 120, and 48% of subjects showed an increment in PMNs at the end of the 4-month observation period. These results are in agreement with previous observations both in rats and humans (*14–16*) that exposures to O_3 > 0.12 ppm (the current U.S. Air Quality Standard for

O_3) induce an inflammatory nasal response. Because SWMMC inhabitants are exposed an average of 4.27 hr/day/359 days a year (1991 data) to levels of O_3 > 0.11 ppm, there is absolutely no possibility of a decrease in the number of PMNs in their upper and lower respiratory airways. If we further observe that the development of nasal epithelial phenotypic changes does not require continuous O_3 exposure (*14*), we have the appropriate conditions for the development of hyperplastic, metaplastic, and dysplastic nasal lesions. The high correlation coefficient between nasal dysplasia and PMNs is an interesting but not surprising finding in view of the previous suggestion that release of neutrophil products is an inductive mechanism for secretory cell hyperplasia and metaplasia in O_3-exposed macaque nasal epithelium (*17*) and the considerable evidence that liberation or generation of oxygen free radicals contribute to carcinogenesis (*9,18–26*). Dysplastic nasal mucosa lesions in SWMMC inhabitants may well be a manifestation of a process of adaptation to prolonged exposure to environmental atmospheric pollutants.

Farber and Rubin (*27*) make several points that could be relevant to this study: carcinogenic agents produce heritable changes in a high proportion of treated cells at both the initiation and promotion stages, although its obvious manifestations (e.g., focus-forming capacity, benign tumors) occur with low probability. Transformation represents an adaptative response to growth constraints and occurs only under specific physiological conditions; the optimal condition for transformation *in vivo* being growth stimulation.

Given the evidence of the rapid development of nasal dysplasia upon arrival in SWMMC (*1*), the question arises how these pathological changes might be elicited and what role ozone plays. Farber and Rubin (*27*) state that "the persistent perturbation of the concentration or localization in cells of proteases and other hydrolytic enzymes might result in a transmissible defect of growth regulation." In this regard, phagocytic cell activation and the resultant host of mediator substances (including proteases, oxidants, neuropeptides, etc.) are both markers and effectors of toxin-induced respiratory tract injury (*15,16,22*).

We propose that repeated, chronic exposures to O_3 and other toxic and potential carcinogenic atmospheric pollutants in SWMMC trigger a series of adaptative responses in the nasal mucosa, characterized histologically by severe loss of normal mucociliary epithelium, basal cell hyperplasia, squamous metaplasia, focal atrophy of the mucosa, and atrophy of submucosal glands [with a consequent decrease in mucus and antioxidant protection (*28*)], submucosal vascular proliferation and telangiectatic blood vessels [the result, perhaps, of the activation of type C sensory nerves and the release of neuropeptides (*22*)], activation of monocytes and macrophages in the submucosa, and mild to severe dysplastic changes, with nasal dysplasia highly correlated with the increment of PMNs in the nasal cavity.

Subjects newly arrived in SWMMC rapidly display these pathological changes, and in less than 1 year they can be included in cytological dysplasia groups. These groups, far from being static, undergo relatively rapid changes, some toward a higher degree of atypia (two severe dysplasias were diagnosed during the 4-month observation period), others toward an apparent reversibility of the phenotypic changes. Although the cytological dysplasia groups seem to be well characterized, the application of the theoretical dysplasia equation reveals that some apparently phenotypically "nonatypical" nasal mucosas are at high risk of developing atypical, dysplastic lesions at a later time.

The theoretical dysplasia equation has been developed to encourage a systematic clinical and cytological approach for analysis and presentation of risk estimates in nasal samples taken from a well-characterized SWMMC population exposed to photochemical smog. Long-term follow-up of these populations will be necessary to establish the real value of the equation and several other elements ought to be added: exposures, doses, and lifetime individual risk of various indoor and outdoor air pollutants of a carcinogenic and toxic nature (*29*).

The possible outcomes of the cytological nasal findings described in this population remain unclear at this point. The spectrum of cytological and histological abnormalities in the nasal turbinates varies from benign reactive changes to lesions characterized by nuclear abnormalities such as enlargement, hyperchromasia, and disturbances of mitotic activity. We have already encountered severe dysplasias and in one case an squamous cell carcinoma *in situ;* however, the incidence of nasal squamous cell carcinoma remains low, at least in the reported cases to oncological hospitals in Mexico City. We suspect that this incidence could increase in the next few years if the problem of urban pollution continues to be severe.

Finally, although ozone is the focus of our investigation, other toxic and carcinogenic substances in the SWMMC may play a role in the nasal mucosa changes described here. In particular, formaldehyde can produce proliferative reactions such as hyperplastic-metaplastic lesions in xenotransplanted human nasal respiratory epithelium (*30*) and squamous cell carcinomas in the rat nasal cavity (*31*). Further work is urgently needed to determine the effect of environmental pollutants in SWMMC inhabitants. Well-characterized groups of subjects should be monitored and have the benefit of a close follow-up of nasal mucosa changes.

References

1. Calderon-Garcidueñas L, Osorno-Velazquez A, Bravo-Alvarez H, Delgado-Chavez R, Barrios-Marquez R. Histopathological changes of the nasal mucosa in southwest metropolitan Mexico City inhabitants. Am J Pathol 140: 225–232(1992).
2. Bertram J, Kolonel L, Meyskens F. Rationale and strategies for chemoprevention of cancer in humans. Cancer Res 47:3012–3031(1987).
3. Stich HF, Hornby AP, Mathew B, Sankaranarayanan R, Krishnan Nair M. Response of oral leukoplakias to the administration of vitamin A. Cancer Lett 40:93–101(1988).
4. Stich HF, Brunnemann KD, Mathew B, Sankaranarayanan R, Krisnan Nair, M. Chemopreventive trials with vitamin A and B-carotene: some unresolved issues. Prev Med 18:732–739(1989).
5. Stich HF, Mathew B, Sankaranarayanan R, Krishnan Nair M. Remission of precancerous lesions in the oral cavity of tobacco chewers and maintenance of the protective effect of B-carotene or vitamin A. Am J Clin Nutr 53: 2985–3045(1991).
6. Mathews-Roth MM. Antitumor activity of ß-carotene, canthaxanthin and phytoene. Oncology 39:33–37(1982).
7. Burton GW, Ingold, KU. Vitamin E as an in vitro and in vivo antioxidant. Ann NY Acad Sci 570:7–22(1989).
8. Elsayed NW, Kass R, Mustafa MG, Hacker AD, Ospital JJ, Chow CK, Cross CE. Effect of dietary vitamin E level on the biochemical response of rat lung to ozone inhalation. Drug-Nutr Interact 5:373–386(1988).
9. Floyd RA. Role of oxygen free radicals in carcinogenesis and brain ischemia. FASEB J 4: 2587–2597(1990).
10. Borek C, Zaider M, Ong A, Mason H, Witz G. Ozone acts alone and synergistically with ionizing radiation to induce in vitro neoplastic transformation. Carcinogenesis 7:1611–1613 (1986).
11. Yokoyama E, Frank R. Respiratory uptake of ozone in dogs. Arch Environ Health 25: 132–138(1972).
12. Johnson NF, Hotchkiss JA, Harkema JR, Henderson RF. Proliferative responses of rat nasal epithelia to ozone. Toxicol Appl Pharmacol 103:143–155(1990).
13. Harkema JR, Plopper CG, Hyde DM, Wilson DW, St. George JA, Wong VJ. Nonolfactory surface epithelium of the nasal cavity of the Bonnet monkey: a morphologic and morphometric study of the transitional and respiratory epithelium. Am J Anat 180:266–279(1987).
14. Hotchkiss JA, Harkema JR, Henderson RF. Effect of cumulative ozone exposure on ozone-induced nasal epithelial hyperplasia and secretory metaplasia in rats. Exp Lung Res 15:589-600(1991).

15. Hotchkiss JA, Harkema JR, Sun JD, Henderson RF. Comparison of acute ozone-induced nasal and pulmonary inflammatory responses in rats. Toxicol Appl Pharmacol 98:289–302(1989).
16. Koren HS, Hatch GE, Graham DE. Nasal lavage as a tool in assessing acute inflammation in response to inhaled pollutants. Toxicology 60:15–25(1990).
17. Harkema JR, Plopper CG, Hyde DM, St George JA,Wilson DW, Dungworth DL. Response of the macaque nasal epithelium to ambient levels of ozone; a morphologic and morphometric study of the transitional and respiratory epithelium. Am J Pathol 128:29–43(1987).
18. Cerutti PA. Prooxidant states and tumor promotion. Science 27:375–381(1985).
19. Cerutti PA. Response modification in carcinogenesis. Environ Health Perspect 81:39–43 (1989).
20. O'Connell JF, Klein-Szanto AJP, Di Giovanni DM, Fries JW, Slaga TJ. Enhanced malignant progression of mouse skin tumors by the free radical generator benzoyl peroxide. Cancer Res 46:2863–2865(1986).
21. Weitzman SA, Weitberg AB, Clark EP, Stossel TP. Phagocytes as carcinogens: malignant transformation produced by human neutrophils. Science 227:1231–1233(1985).
22. Cross CE, Halliwell B. Biological consequences of general environmental contaminants. In:The lung: scientific foundations (Crystal RG, West JB, eds). New York:Raven Press, 1991; 1961–1973.
23. Goldstein BD, Witz G. Free radicals and carcinogenesis. Free Rad Res Commun 11:3–10 (1990).
24. Stich HF, Anders F The involvement of reactive oxygen species in oral cancers of betel quid/tobacco chewers. Mutat Res 214:47–61(1989).
25. Simic MG, Bergtold DS, Karam LR. Generation of oxy radicals in biosystems. Mutat Res 214:3–12(1989).
26. Goldstein BD, Czerniecki B, Witz G. The role of free radicals in tumor promotion. Environ Health Perspect 81:55–57(1989).
27. Farber E, Rubin H. Cellular adaptation in the origin and development of cancer. Cancer Res 51:2751–2761(1991).
28. Cross CE, Halliwell B, Allan A. Antioxidant protection: a function of tracheobronchial and gastrointestinal mucus. Lancet i:1328–1330 (1984).
29. Naugle DF, Pierson TK. A framework for risk characterization of environmental pollutants. J Air Waste Manag Assoc 41:1298–1307(1991).
30. Klein-Szanto AJP, Ura H, Resau J. Formaldehyde-induced lesions of xenotransplanted human nasal respiratory epithelium. Toxicol Pathol 17:33–37(1989).
31. Kerns WD, Pavkov KL, Donotrio DJ, Gralla EJ, Swenberg, JA. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res 43:4382–4392 (1983).